# Hordoindolines are associated with a major endosperm-texture QTL in Barley (*Hordeum vulgare*)

**B. Beecher, J. Bowman, J.M. Martin, A.D. Bettge, C.F. Morris, T.K. Blake, and M.J. Giroux**

**Abstract**: Endosperm texture has a tremendous impact on the end-use quality of wheat (*Triticum aestivum* L.). Cultivars of barley (*Hordeum vulgare* L.), a close relative of wheat, also vary measurably in grain hardness. However, in contrast to wheat, little is known about the genetic control of barley grain hardness. Puroindolines are endosperm-specific proteins found in wheat and its relatives. In wheat, *puroindoline* sequence variation controls the majority of wheat grain texture variation. Hordoindolines, the puroindoline homologs of barley, have been identified and mapped. Recently, substantial allelic variation was found for hordoindolines among commercial barley cultivars. Our objective was to determine the influence of hordoindoline allelic variation upon grain hardness and dry matter digestibility in the 'Steptoe' × 'Morex' mapping population. This population is segregating for hordoindoline allele type, which was measured by a *HinA*/*HinB*/*Gsp* composite marker. One-hundred and fifty lines of the 'Steptoe' × 'Morex' population were grown in a replicated field trial. Grain hardness was estimated by near-infrared reflectance (NIR) and measured using the single kernel characterization system (SKCS). Variation attributable to the *HinA*/*HinB*/*Gsp* locus averaged 5.7 SKCS hardness units (SKCS U). QTL analysis revealed the presence of several areas of the genome associated with grain hardness. The largest QTL mapped to the *HinA*/*HinB*/*Gsp* region on the short arm of chomosome 7 (5H). This QTL explains 22% of the SKCS hardness difference observed in this study. The results indicate that the *Hardness* locus is present in barley and implicates the hordoindolines in endosperm texture control.

*Key words*: puroindolines, grain hardness, digestibility.

**Résumé** : La texture de l'albumen a un impact considérable sur la qualité du blé selon les différentes utilisations projetées. Les cultivars de l'orge (*Hordeum vulgare* L.), un proche parent du blé (*Triticum aestivum* L.), montrent aussi une grande variation pour ce qui est de la dureté du grain. Cependant, contrairement à la situation chez le blé, bien peu de choses sont connues quant au contrôle génétique de la dureté du grain chez l'orge. Les puroindolines sont des protéines spécifiques de l'albumen qu'on retrouve chez le blé et les espèces voisines. Chez le blé, la variation de la séquence de *puroindolines* détermine largement la variation quant à la texture des grains. Les hordoindolines, les homologues des puroindolines chez l'orge, ont été identifiées et cartographiées. Récemment, une importante variation allélique au niveau des hordoindolines a été observée chez des cultivars commerciaux de l'orge. L'objectif des auteurs était de déterminer l'impact de la variation allélique au niveau des hordoindolines sur la dureté des grains et la digestibilité de la matière sèche au sein de la population de cartographie dérivée du croisement 'Steptoe' × 'Morex'. Cette population est en ségrégation pour le type d'hordoindoline, lequel a été déterminé à l'aide du marqueur composite *HinA*/*HinB*/*Gsp*. Cent cinquante lignées de la population 'Steptoe' × 'Morex' ont été cultivées en parcelles d'essai. La dureté des grains a été mesurée par réflectivité en proche infrarouge (NIR) à l'aide d'un seul grain (SKCS : « single kernel characterization system »). La variation attribuable au locus *HinA*/*HinB*/*Gsp* était en moyenne de 5,7 unités de dureté SKCS. Une analyse QTL a révélé l'association de plusieurs régions génomiques avec la dureté des grains. Le QTL le plus important était localisé dans la même région que le locus *HinA*/*HinB*/*Gsp* sur le bras court du chromosome 7 (5H). Ce QTL permet d'expliquer 22 % des différences de dureté des grains observées lors de cette étude. Ces résultats indiquent que

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le locus *Hardness* est présent chez l'orge et que les hordoindolines seraient impliquées dans le contrôle de la texture de l'albumen.

*Mots clés* : puroindolines, dureté des grains, digestibilité.

[Traduit par la Rédaction]

## Introduction

Improvement of grain quality is an important part of cereal crop improvement efforts. Substantial resources have been devoted to mapping and characterizing the genes responsible for variation in barley (*Hordeum vulgare* L.) grain quality. DNA mapping efforts have identified regions of chromosomes associated with quantitative traits such as malting or feed quality (Hayes et al. 1993; Rouvès et al. 1996). The genetic control of grain texture in barley has received little attention, although there is evidence to suggest that it is important. Milling energy, a measure of grain texture, appears to correlate negatively with barley malting quality (Allison 1986; Brennan et al. 1996). In addition, it has been shown that endosperm particle size affects digestion in ruminants (Bowman et al. 2001). Significant advances in selection for both malting and feed quality may therefore depend upon the grain textural differences inherent to barley. In other members of the Triticeae, such as wheat (*Triticum aestivum* L.), grain textural differences have traditionally been a major factor in selection of new varieties owing to their large impacts on end-product quality. Because wheat grain texture is one of the primary determinants of end-product quality, hardness is a major market-class distinction for wheat (i.e.wheat is marketed as either hard or soft.)

Endosperm hardness can be measured in many ways. Traditionally, wheat hardness has been measured by the particle size index (PSI) or near-infrared reflectance (NIR), whereas the measurement of barley hardness has mainly been limited to milling energy (Symes 1965; Norris et al. 1989). The single kernel characterization system (SKCS), which measures hardness as a function of the force required to crush the whole kernel, can be used for both species and is becoming a popular means of measuring this trait (Gaines et al. 1996; Psotka 1996).

Wheat-grain hardness is simply inherited and controlled primarily by a single locus, termed *Hardness* (*Ha*) (Symes 1965; Baker 1977) that resides on the short arm of chromosome 5D (Mattern et al. 1973; Law et al. 1978). Soft wheats (*Ha*) are physically softer in endosperm texture and produce flours of smaller size and size distribution than do hard wheats (*ha*). Three genes closely linked to *Ha* have been identified (Rahman et al. 1994; Sourdille et al. 1996; Giroux and Morris 1998). These are the structurally related genes *puroindoline a* (*pinA*), *puroindoline b* (*pinB*), and *Gsp-1a*. All hard wheats characterized to date have a sequence alteration in either *pinA* or *pinB* (Giroux and Morris 1998). The most common mutation is a glycine→serine alteration in *pinB*, which is present in the majority of American hard wheats surveyed (Morris et al. 2001). Expression of wild type *pinB* in a hard wheat containing this mutant *pinB* sequence fully restored the soft phenotype (B. Beecher, A. Bettge, and M.J. Giroux, unpublished data). *PinA* and *PinB* expression has also been shown to confer a softer endosperm texture in transgenic rice plants (Krishnamurthy and Giroux 2001). Thus, the evidence is strong that puroindolines are responsible for the function of *Ha* in wheat.

The puroindoline homologs of barley, the hordoindolines, have been identified (Gautier et al. 2000). They map to the short arm of chomosome 7 (5H), the same chromosomal location of puroindolines in wheat (Rouvès et al. 1996; Beecher et al. 2001). A recent study suggests that substantial allelic variation exists in barley for both *hordoindoline a* (*hinA*) and *hordoindoline b* (*hinB*) sequences (Beecher et al. 2001). The chromosomal region in which the *hordoindolines* are found appears to be involved in grain texture dependent traits such as milling energy and level of fine grind extract, as well as malt extract yield (Thomas et al. 1996; Mather et al. 1997; Beecher et al. 2001). Our objective was to identify regions of the genome that affect barley-grain hardness in a QTL analysis. We were also particularly interested in determining whether *hordoindoline* allelic variation influenced endosperm texture and related traits in a doubled haploid population segregating at this locus.

## Materials and methods

### Plant material

The barley mapping population consisted of 150 doubled-haploid lines (DHLs) generated from a 'Steptoe' (CI15229) × 'Morex' (CI15773) cross (Kleinhofs et al. 1993). The 150 lines and parents were grown in randomized trials in Bozeman, Mont., in 1992 and 2000. Seed was bulked from replications grown in the field.

### Seed texture measurement

Barley seed texture was analyzed by both the SKCS 4100 (Perten Instruments, Springfield, Ill.) and NIR InfraAlyzer 400 (Technicon Corp., Tarrytown, N.Y.). NIR analysis (method 39-70A) (AACC 2000) was performed on replicates of approximately 10 g wheat seeds ground into a wholewheat flour on a UDY mill (UDY Co., Fort Collins, Colo.) fitted with a 0.5-mm screen. The SKCS machine was used to estimate hardness, kernel weight (wt.), and kernel diameter. SKCS analysis was performed on samples of 100 seeds.

### Dry-matter digestibility

Many of the doubled haploid lines in the 'Steptoe' × 'Morex' population have poor agronomic characteristics. Dry-matter digestibility (DMD) was performed on 115 lines from the 'Steptoe' × 'Morex' mapping population that produced sufficient grain for all required analyses. Grain samples were cracked using a Buehler mill (Buehler-Miag, Braunschweig, Germany) to simulate the dry-rolling processing done before feeding barley. To measure ruminal DMD (Vanzant et al. 1998), four 5-g samples of each line were weighed and placed into 10 × 20 cm, 50-μm pore poly-

**Fig. 1.** QTL Analysis of the 'Steptoe' × 'Morex' mapping population. QTL scans of barley grain protein percentage (blue trace), grain diastatic power (red trace), heading date (green trace), and grain hardness as measured by the SKCS (black trace). LOD scores reported at left. Dotted line indicates LOD = 2.0. The heading date gene (most likely *Ppd1*) on chromosome 2 exerts so great an effect on phenotype that the QTL scales could not easily be justified for heading date and grain protein percentage. The LOD score scale for heading date is shown in green, whereas that for the other three traits is shown in black. The position of the *HinA/HinB/Gsp* markers on chromosome 7 is noted.

ester bags (Ankom Technology, Fairport, N.Y.). Two of the four polyester bags for each line were placed in the rumen of each of two ruminally cannulated steers and incubated for 3 h. All animals were cared for under guidelines equivalent to those laid down by the Canadian Council on Animal Care. Two additional empty blank bags were included with each incubation to correct for DM content owing to microbial contamination. After removal from the rumen, the bags were rinsed under cold water until the wash water ran clear. The bags were dried at 60°C for 48 h, and then weighed. Dry-matter (DM) content of the cracked barley samples was measured (AOAC 1997). Ruminal DMD (measured in grams per kilogram) was calculated according to the following equation:

$$\text{Dry matter digestibility} = \frac{(\text{sample wt.}_{\text{in}} \times \text{sample DM content}) - (\text{sample wt.}_{\text{out}} - \text{blank}) \times 1000}{(\text{sample wt.}_{\text{in}} \times \text{sample DM content})}$$

### Grain and agronomic quality

Grain protein and heading date were measured in both 1992 and 2000 as described by See et al. (2002). Diastatic power was measured in 1992 only and the data set is available from http://wheat.pw.usda.gov/ggpages/.

### QTL analysis

Phenotype measurements were obtained for both 1992 and 2000 and entry means were merged with the 'Steptoe' × 'Morex' DHL marker dataset available from http://wheat.pw.usda.gov/ggpages/. Linkage maps were reconstructed using Mapmaker 3.0 (Lander et al. 1987) with linkage indicated by a minimum LOD of 4.0 and the maximum distance between linked genes set at 35 cM. In this dataset, no recombination has been observed among the markers *HinA*, *HinB*, and *Gsp*. Therefore, our analysis relied upon a *HinA*/*HinB*/*Gsp* composite *Hardness (Ha)* marker. The *Ha-s* locus is defined as the *HinA*/*HinB*/*Gsp* alleles carried by 'Steptoe' and the *Ha-m* locus is defined as the *HinA*/*HinB*/*Gsp* alleles carried by 'Morex'. The LOD score and percentage of phenotypic variance accounted for by the *Ha* locus was taken directly from the Mapmaker QTL output. We considered LOD scores greater than 3.0 to be likely indicators of QTL.

### Statistical analysis

Analyses of variance were computed for each trait using the environments as replications. The entry's source ofs variation was partitioned by including a fixed effect for hordoindoline class and a random effect for entries within classes using PROC GLM in SAS (SAS Institute Inc. 1988). Differences between hordoindoline class means were tested using the entries within-class mean square. Correlations among traits were computed from entry means.

## Results

### A major QTL for barley grain hardness is located on the short arm of chromosome 7 (5H)

Quantitative trait loci (QTLs) impacting grain hardness could be found on barley chromosomes 1, 4, 5, and 7 (Fig. 1). The chromosome-1 gene region impacting hardness impacted no other measured trait. The *Hardness* gene region on chromosome 4, near ABG319A, reflected the impact of one or more genes modifying grain protein content. The effect of the *Hardness* gene region on chromosome 5 was nearly concordant with variation in diastatic power. The two gene regions on chromosome 7, centered around *HinA*/*HinB*/*Gsp* and CDO504, showed no significant impact on grain protein content, diastatic power, or heading date. From this point forward, merely for readability, we will refer to these chromosomal regions as QTL.

The grain hardness QTL cosegregating with the *HinA*–HinB–*Gsp* gene family accounts for approximately 22% of the total phenotypic variation in grain hardness, whereas the other four QTL indicated account for between 9 and 13%. Two of these QTL appear to impact hardness in a pleiotropic manner, one through gross-grain composition (grain protein percentage) and the other perhaps by regulating the amount of β-*amylase*, a large contributor to overall endosperm protein composition in barley and the primary determinant of diastatic power. The three genes that have no obvious pleiotropic correlants and may be provisionally considered to impact grain hardness in a direct fashion account for approximately 45% of the phenotypic variation for this trait, with the effect centered around the *HinA*/*HinB*/*Gsp* complex responsible for about half of this overall impact. For simplicity, this hordoindoline complex will hereafter be referred to as *Hardness* (*Ha*).

### Hordoindoline allelic state has a significant impact upon both kernel hardness and DMD

The 150 lines of the 'Steptoe' × 'Morex' mapping population segregated 77 *Ha-m* (*Hardness* allele from the 'Morex' parent) : 73 *Ha*-s (*Hardness* allele from the 'Steptoe' parent), or approximately 1:1 ($\chi^2 < 0.2$). Hardness, kernel weight, kernel diameter, and dry-matter digestibility traits were measured for all lines. Dry-matter digestibility was measured on a subset of 115 lines where sufficient grain was available. SKCS hardness values ranged from 37.2 to 76.7 units, whereas NIR-estimated hardness values ranged from –33.6 to –6.3 units. The correlation between NIR-estimated hardness and hardness directly measured by SKCS was significant, but weak (0.24, Table 1). Kernel weight ranged from 30.1 to 48.6 mg and kernel diameter ranged

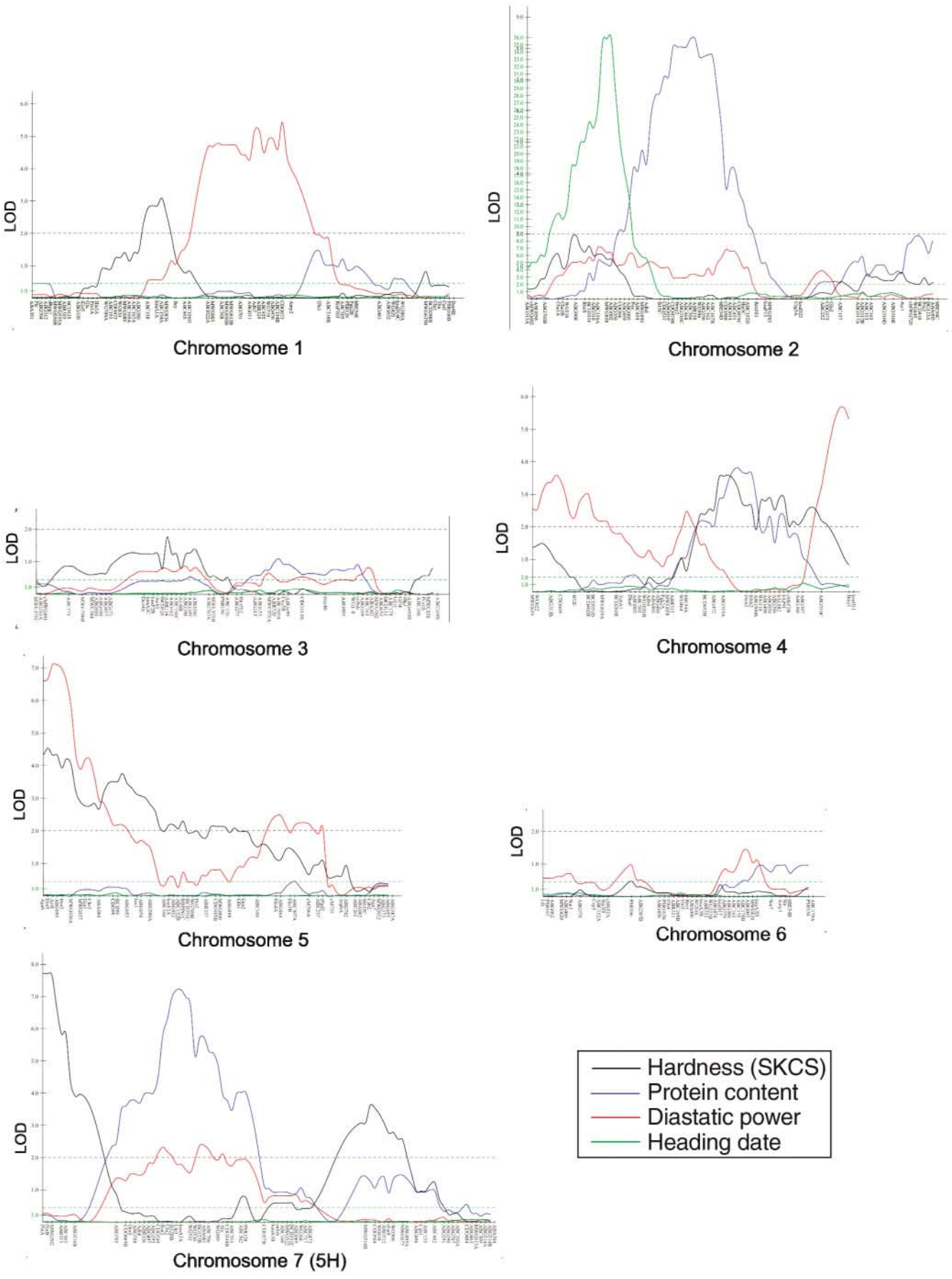


**Table 1.** Correlations among grain hardness, kernel weight, kernel diameter, and dry-matter digestibility where *hordoindoline* allele makeup differed for doubled haploid lines from 'Steptoe' × 'Morex'.

| Trait | SKCS[†] hardness | NIR[‡] hardness | Kernel wt. | Kernel diameter |
|---|---|---|---|---|
| NIR hardness | 0.24** | | | |
| Kernel wt. | –0.38** | –0.20* | | |
| Kernel diameter | –0.36** | –0.17* | 0.85** | |
| Dry-matter digestibility | –0.37** | 0.11 | 0.19* | 0.18* |

**Note:** *, significant at the 0.05 probability level; **, significant at the 0.01 probability level.
[†]Single-kernel characterization system.
[‡]Near-infrared reflectance.

from 1.88 to 2.64 mm. DMD ranged from 27.1 to 52.3%. A significant negative correlation was found between dry-matter digestibility and kernel hardness (0.37, Table 1).

Significant differences were found between *Hardness* allelic classes for all measured traits (Table 2). The group containing the *Ha-m* alleles averaged 63.0 SKCS hardness units, whereas the group containing the *Ha-s* alleles averaged 57.3 SKCS hardness units. The difference between the two classes was 5.7 SKCS U, with the harder phenotype associated with the *Ha-m* allele. NIR hardness values differed by 3.1 NIR U in the same manner. The harder *Ha-m* class had smaller average kernel weight (37.6 vs. 38.9 mg) and diameter (2.26 vs. 2.32 mm) than the softer *Ha-s* class. Kernel weight and diameter differed by 1.3 mg and 0.06 mm, respectively, the larger values associating with the *Ha-s* allele. Dry-matter digestibility showed a similar trend. Allelic classes differed by 1.9%; 39.2 and 41.1% for *Ha-m* and *Ha-s*, respectively.

The distribution of lines according to hordoindoline type is presented for both hardness and dry-matter digestibility. SKCS hardness showed a bimodal distribution with the harder *Ha-m* class having a greater range and completely overlapping the softer *Ha-s* class (Fig. 2). Distribution for dry-matter digestibility was also bimodal, but the range within the hordoindoline classes was similar (Fig. 3).

### Correlations among measured traits

Correlation analysis was performed for all trait combinations (Table 1). The SKCS and NIR methods of measuring grain hardness exhibited a positive, but poor, correlation ($r$ = 0.24). This likely reflects the fact that grain hardness in barley has not been well studied and that the calibration for NIR hardness used was based on a set of wheat genotypes varying in grain hardness. SKCS hardness correlated negatively with kernel weight ($r$ = –0.38), kernel diameter ($r$ = –0.36), and dry matter digestibility ($r$ = –0.37), and NIR-estimated hardness correlated weakly and negatively with kernel weight ($r$ = –0.20) and kernel diameter ($r$ = –0.17). As expected, kernel weight correlated well with diameter ($r$ = 0.85). The relationship between SKCS Hardness, digestibility, and *hordoindoline* allele type was represented graphically (Fig. 4). Lines carrying the *Ha-m* allele tend to have higher hardness values and lower digestibility values (Fig. 4), whereas lines carrying *Ha-s* tended to have lower hardness values and higher digestibility values.

## Discussion

QTL analysis was performed on the 'Steptoe' × 'Morex' mapping population to determine which regions of the genome contribute to endosperm texture. The most significant QTL for hardness in this study was located on the distal end of the short arm of chromosome 7 (5H), coincident with the location of the *HinA–HinB–Gsp* markers (Kleinhofs et al. 1993; Rouvès et al. 1996; Beecher et al. 2001). This chromosomal region has previously been implicated in explaining a portion of the level of fine-grind extract and extract viscosity differences (Mather et al. 1997). This location coincides with the *Hardness* locus of wheat that resides on the short arm of chromosome 5D (Mattern et al. 1973; Law et al. 1978). Grain texture has been well studied in wheat, barley's close relative. In wheat, the *puroindoline* genes are associated with the majority of grain-hardness variation (Giroux and Morris 1998). Recent evidence indicates that the puroindolines directly control rice and wheat grain texture (Krishnamurthy and Giroux 2001; B. Beecher, A. Bettge, and M.J. Giroux, unpublished data). They appear to accomplish this by physically interacting with the surface of the starch granule (Greenwell and Schofield 1986). Like puroindolines, hordoindolines have also been found on the surface of barley starch granules (Greenwell and Schofield 1986; Darlington et al. 2000). It seems likely that these homologous sequences control endosperm texture to a large degree in both wheat and barley.

Barley cultivars differ in traits related to grain texture. A survey of barley cultivars found that milling energy, another measure of grain hardness, correlates negatively with malting quality (Allison 1986; Brennan et al. 1996). This study indicates that SKCS hardness is negatively correlated with percentage dry-matter digestibility by ruminants. However, larger particles and slower dry-matter digestibility is associated with higher feed quality (Bowman et al. 2001). Therefore, the development of softer cultivars may benefit malting-quality traits and the development of harder barleys may benefit feed-quality traits. However, until now, the genetic control of barley grain hardness has not received much attention. Perhaps this is because barley has been thought to exhibit relatively little texture variation. In fact, barleys appear to behave in a similar fashion to hard hexaploid wheat. That is, the barley lines in this study averaged 60.3 SKCS U, placing them within the hard range of wheat varieties. The average SKCS hardness difference between lines varying in *hordoindoline* allele types was 5.7, similar to the average difference of 6 SKCS hardness units reported in a similar study involving various *Hardness* alleles found in hard wheats (Giroux et al. 2000). The average hardness difference, which is conferred by *hordoindoline* allele type, is similar to that reported among the different alleles of *Hardness* in hard wheat (Martin et al. 2001; Lillemo and Morris 2000).

The differences between the SKCS hardness assay and NIR estimates of hardness appear largely because of the wheat-based hardness calibration used for the NIR estimates

**Table 2.** *Hordoindoline* allele class means, range, and parent means for grain hardness, kernel morphology traits, and dry matter digestibility of the 'Steptoe' × 'Morex' doubled-haploid population.

| | Grain hardness | | | | |
|---|---|---|---|---|---|
| Entry | SKCS[†] | NIR[‡] | Kernel wt. (mg) | Kernel diameter (mm) | Dry-matter digestibility (%) |
| *Ha-m* | 63.0 | –20.4 | 37.6 | 2.26 | 39.2 |
| *Ha-s* | 57.3** | –23.3** | 38.9** | 2.32** | 41.1* |
| Range | 37.2–76.7 | –33.6–(6.3) | 30.1–48.6 | 1.88–2.64 | 27.1–52.3 |
| 'Morex' | 53.6 | –24.1 | 38.7 | 2.41 | 32.7 |
| 'Steptoe' | 56.1 | –25.6 | 42.0 | 2.39 | 36.5 |
| CV% | 6.2 | 23 | 6.3 | 5.0 | 19 |

**Note:** Date is based on the mean from two years.*, difference between hordoindoline class means is significant at the 0.05 probability level; **, difference between hordoindoline class means is significant at the 0.01 probability level.
[†]Single-kernel characterization system.
[‡]Near-infrared reflectance.

**Fig. 2.** Distribution of 77 lines within the *Ha-m* class and 73 lines within the *Ha-s* class for SKCS hardness from the 'Steptoe' (*Ha-s* allele) × 'Morex' (*Ha-m* allele) doubled haploid mapping population. SKCS average hardness values shown on the *x* axis. The *y* axis denotes number of individuals per hardness class. White bars represent individuals that contain the *Ha-m* allele. Black bars represent individuals carrying the *Ha-s* allele. Two-year means reported.

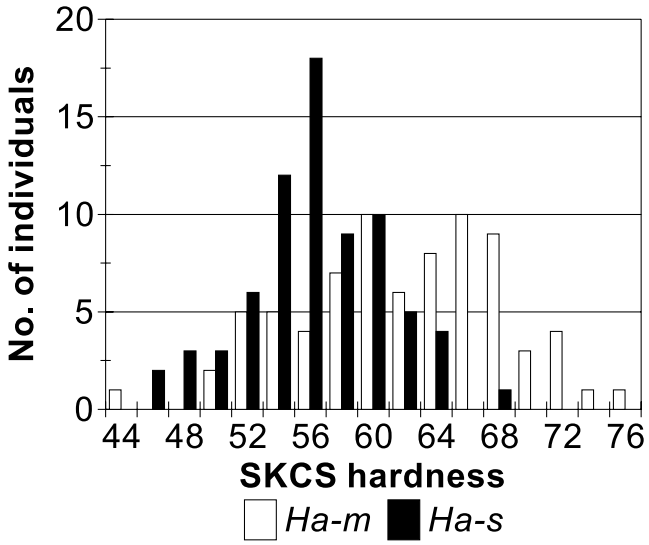
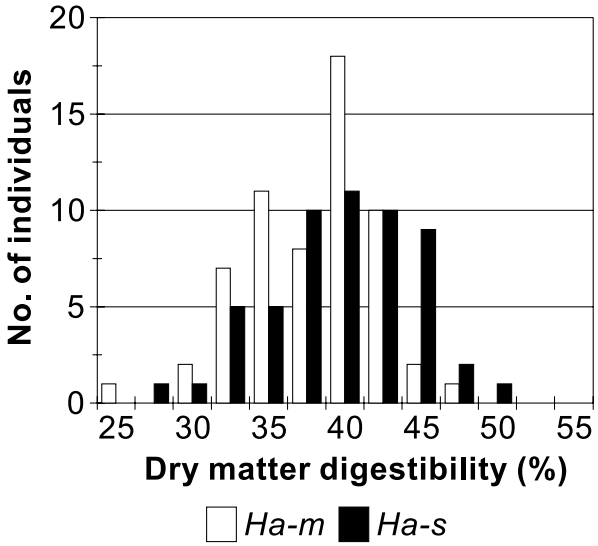


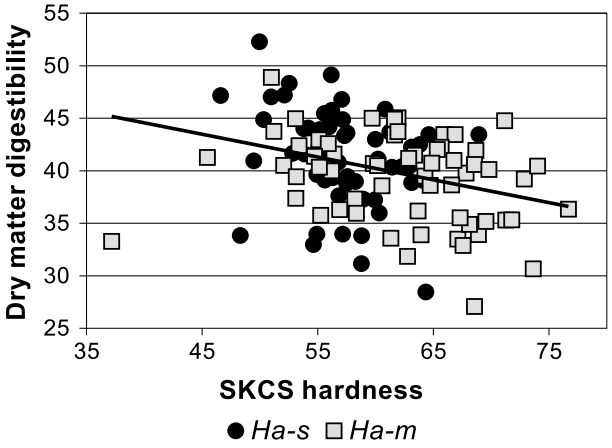

**Fig. 3.** Distribution of 77 lines within the *Ha-m* class and 73 lines within the *Ha-s* class for dry matter digestibility (DMD) percentage from the 'Steptoe' (*Ha-s* allele) × 'Morex' (*Ha-m* allele) doubled-haploid mapping population. Dry matter digestibility values shown on the *x* axis. The *y* axis denotes number of individuals per digestibility class. White bars represent individuals that contain the *Ha-m* allele. Black bars represent individuals carrying the *Ha-s* allele. Two-year means are reported.




(AACC 2000). If market-class segregation (malting vs. feeding) is to be done for barley at the elevator, a better NIR calibration for barley-kernel hardness should be developed.

Thus, it appears that barley cultivars may be analogous to hard wheat cultivars. That is, barley cultivars exhibit small but significant amounts of variation in grain hardness that affect grain quality, which is controlled to some extent by the *Hardness* locus. The small differences between the *hordoindoline* sequences of barley and their soft-wheat homologs may be important in this context (Beecher et al. 2001). Some of these amino-acid sequence changes might influence the degree to which hordoindolines function in grain texture. A difference of as little as one amino acid has been shown to greatly decrease the function of puroindolines in reducing grain hardness (Giroux and Morris 1997; B. Beecher, A. Bettge, and M.J. Giroux, unpublished data). The changes in the *hordoindoline* sequences of barley relative to those in soft wheats may be fixed in the species, and therefore the natural potential for softness may be less than for wheat. The diploid progenitors of hexaploid wheat are soft textured (Williams 1986). However, that does not appear to be the case for barley. A survey of several populations of wild barley (*Hordeum spontanaeum*) found significant variability in grain hardness as measured by milling energy (Ellis et al. 1993). Interestingly, the observed variability in the wild material ranged towards the hard end of the observed variation for domestic cultivars. Other research has included a wider range of cultivated germplasm in barley grain-hardness studies. Thus far, no barley varieties have been reported as having endosperm texture typical of soft

**Fig. 4.** Relationship between dry-matter digestibility and SKCS hardness for 150 lines from the 'Steptoe' (*Ha-s* allele) × 'Morex' (*Ha-m* allele) doubled-haploid mapping population. SKCS hardness values shown on the *x* axis. Percentage DMD shown on the *y* axis. Two-year means are reported.




wheats. In addition, although friabilin has been observed in barley, the amounts present are smaller than those observed in wheat (Jagtap et al. 1993; Morrison et al. 1992). Smaller amounts of friabilin, HinA, and HinB on the starch surface would be expected in barley if HinA and (or) HinB were largely nonfunctional.

This study has identified a major QTL for endosperm hardness in barley, along with strong gene candidates for hardness control. These findings may prove to be useful tools in barley improvement efforts. This and other studies suggest that the development of barley cultivars varying widely in endosperm texture may be desirable for both feed and malting purposes (Allison 1986; Brennan et al. 1996; Bowman et al. 2001). Correlations among SKCS hardness, dry-matter digestibility, kernel weight, and kernel diameter were relatively small, but significant. Measurement of traits such as malting quality and digestibility is difficult and time-consuming. If further studies confirm the relationship between these traits and the easily meassured SKCS grain hardness, future grain improvement efforts would greatly benefit. Screening of barley accessions by SKCS could lead to the discovery of additional sources of texture variation, which could be easily incorporated into elite germplasm by marker-assisted selection. However, if a natural source is not found, the similarity between barley and wheat suggests that barley endosperm texture could be modified by transformation, as has already been demonstrated in wheat (Beecher et al. 2001; B. Beecher, A. Bettge, and M. J. Giroux, unpublished data). Expression of a wheat puroindoline sequence in barley could increase softness beyond that which is presently available. The results of such a study would be interesting, because increased grain softness here correlated with increased dry-matter digestibility.

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